Cloning and Expression of Pax8 from Adult Kidney of the Little Skate, *Leucoraja erinacea*

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Previously, we have shown that the adult kidney of the little skate *Leucoraja erinacea* possesses a nephrogenic zone that resembles the embryonic mammalian metanephric kidney[1]. The cell types of the elasmobranch and the mammalian nephrons are highly similar[2] and this nephrogenic zone contains the essential structures characteristically found in the embryonic mammalian metanephros. Therefore, we hypothesized that the same genes critical in mammalian embryonic kidney development also play a role in the ongoing nephrogensis of the adult skate kidney. As nephrons form in the kidney they express critical transcription factors such as WT1, Pax2 and Hoxa11 which condense and secrete Wnt4. Wnt4 is required for nephrogenesis to continue. Wnt4 acts as an autocrine loop to stimulate its own synthesis and is required for cells to differentiate into epithelia[3]. The homeobox gene Pax8, is also expressed in the condensed mesenchyme and S-shaped bodies of the forming ureteric bud and may be responsible for glomeruli maturation[4]. Pax8 has also been linked to co-transcription factors to induce pronephric tubule differentiation and growth[5]. The aforementioned genes Pax2 and Wnt4 have been fully characterized and our previous research has shown their expression in the developing niche of adult skate kidneys, demonstrating that the roles of these genes were conserved through evolution. However, there has been no molecular characterization of Pax8 expression in adult skate kidneys.

The original 827 base pair Pax8 sequence originated from a pooled organ library of *Leucoraja erinacea* and was found in the marine genomics database (http://www.marinegenomics.org). Primer walking was utilized to retrieve additional length of the initial sequence. Running in the 3' to 5' direction an 18bp primer GCTGAGTACAAGCGGCAG was designed to sit at the 564bp site. An additional 410bp were retrieved after sequencing at the MDIBL DNA Sequencing Facility. Primer walking was repeated at the new 1,237bp site with a 27bp primer GCCAAGCCCAGTTACACATCATCTGCC. After sequencing, an additional 870bp were retrieved, resulting in a final sequence length of the 2,175 bp.

Using BLAST search algorithms (www.ncbi.nlm.nih.gov/BLAST), the Pax8 2,175bp sequence was analyzed and compared with database entries for homology. Pax8 homologues of *Homo sapiens*, *Mus musculus*, and *Xenopus laevis* were found and aligned with the Pax8 homologue from *Leucoraja erinacea* using MultAlin (http://prodes.toulouse.inra.fr/multalin/multalin.html).

Because the Pax8 gene originated from a normalized library of combined *Leucoraja erinacea* organs, the organ of origin was uncertain. Relative expression levels of Pax8 in various skate organs were determined using Real-Time Quantitative PCR. Skate kidney, brain, muscle, spleen, rectal gland, and heart tissue were obtained. RNA from each was extracted using Trizol (Invitrogen) as per manufacturer's specifications. After a DNase-digest 1 μg of total RNA was reversed transcribed using random hexamers, poly (dT)-oligonucleotides and M-MLV reverse transcriptase (Invitrogen).

Real-time qPCR oligonucleotide primers were designed using Primer Express software. The forward primer had a sequence of TGCGGCCTTGTGACATCTC, the reverse primer sequence was TGCCAAGGATTTTGCTGACA. Expression levels of Pax8 in the various organs were determined using SYBR- green chemistry. For normalization, b-actin expression was measured. The relative mRNA expression was analyzed using qgene software[6].

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          321                                                                 400
Leucoraja TCGAATCAAT CAACTCGGAG GCATGTTTGT GAATGGGAGA CCGCTTCCCG ATGTGGTGAG GCAGAGGATT GTCGACCTTG
    Homo  AGGGCTGAAC CAGCTGGGAG GGGCCTTTGT GAATGGCAGA CCTCTGCCGG AAGTGGTCCG CCAGCGCATC GTAGACCTGG
    Mus   AGGGCTGAAT CAACTAGGAG GGGCCTTTGT GAATGGCAGG CCTCTGCCAG AAGTTGTACG TCAACGCATT GTGGACTTGG
  Xenopus AGGTTTGAAT CAGTTAGGTG GGGCTTTTGT GAATGGACGA CCTCTACCAG AGGTGGTGAG GCAGCGGATT GTTGACCTGG

          401                                                                 480
Leucoraja CACACCAAGG GGTGCGGCCT TGTGACATCT CACGCCAGCT CAGGGTCAGT CACGGGTGTG TCAGCAAAAT CCTTGGCAGG
    Homo  CCCACCAGGG TGTAAGGCCC TGCGACATCT CTCGCCAGCT CCGCGTCAGC CATGGTTGCG TCAGCAAGAT CCTTCGCAGG
    Mus   CCCACCAGGG CGTGAGGCCC TGTGATATTT CTCGCCAGCT CCGTGTCAGC CATGGCTGTG TAAGCAAGAT CCTTGGCAGG
  Xenopus CGCACCAGGG GGTACGTCCC TGTGACATCT CACGACAGCT CAGAGTCAGT CATGGCTGTG TCAGCAAAAT TTTGCGCAGG

          481                                                                 560
Leucoraja TACTATGAAA CCGGCAGCAT CATGCCAGGA GTCATCGGGG GCTCAAAGCC AAAGGTTGCC ACGCCAACAG TGGTGGAGAA
    Homo  TACTACGAGA CTGGCAGCAT CCGGCCTGGA GTGATAGGGG GCTCCAAGCC CAAGGTGGCC ACCCCCAAGG TGGTGGAGAA
    Mus   TACTACGAGA CTGGCAGCAT CCGGCCTGGA GTGATAGGGG GCTCCAAGCC CAAGGTGGCC ACCCCCAAGG TGGTGGAGAA
  Xenopus TACTATGAGA CAGGCAGTAT CCGGCCAGGT GTCATTGGAG GTTCCAAGCC CAAGGTTGCC ACCCCCAAAG TGGTGGAGAA

          561                                                                 640
Leucoraja GATACCTCAG TACAAGCCGC AGAACCCGAC AATGTTTGCG TGCGAGATCA GAGACAGGCT GCTGGCAAAA GGAGTGTGTA
    Homo  GATTCGGGAC TACAAACGCC AGAACCCTAC CATGTTTGCC TGCGAGATCC GAGACCGGCT CCTGGCTGAG GGCGTCTGTG
    Mus   GATACGAGAC TACAAGCGGC AGAACCCTAC CATGTTTGCT TGCGAGATCC GGGACCGGCT CCTGGCAGAA GGCGTTTGTG
  Xenopus AATCGGAGAT TACAAACGCC AGAACCCAAC AATGTTTGCC TGCGAAATCA GGGACCGGCT GCTGACAGAC GGGGTGTGCG

          641                                                                 720
Leucoraja ATAATGATAC TGTGCCCAGC GTCAGCTCCA TAAACAGAAT TATAAGAACC AAAGTTCACA GCCATTNCAT CTACCTCTTG
    Homo  ACAATGACAC TGTGCCCAGT GTCAGCTCCA ITAATAGAAT CATCCGGACC AAAGTGCAGC AACCATTCAA CCTCCCTATG
    Mus   ACAATGACAC TGTCCCCAGT GTCAGCTCCA TCAACAGAAT CATCCGGACC AAAGTGCAGC AGCCATTCAA CCTCCCCATG
  Xenopus ACAATGACAC AGTTCCCAGT GTCAGCTCTA TCAACAGAAT CATACGCACT AAAGTACAGC AACTTTTTAA CCTGCCCATG
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Fig 1. Amino acid sequence of Pax8 from *Leucoraja erinacea, Homo sapiens, Mus musculus*, and *Xenopus laevis* were aligned using MultAlin. Areas of high consensus are seen in gray, areas of low consensus are seen in black.

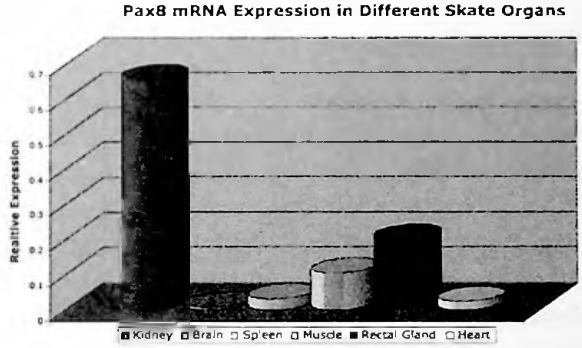

Fig 2. Distribution of Pax8 transcript in different organs from adult skate. Total RNA from kidney, brain, spleen, muscle, rectal gland and heart was extracted, reverse transcribed and applied to qPCR using SYBR-green chemistry. For normalization expression of skate β-actin expression was measured.

Pax8 showed high expression in kidney and relatively low expression in other organs. Most likely the original Pax8 sequence was derived from the skate kidney. These data also showed that the developmentally regulated gene Pax8 displayed high levels of consensus between skate and other species. This supports our hypothesis that the same genes critical in mammalian embryonic kidney development also play a role in the ongoing nephrogensis of the adult skate kidney.

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